NATIONAL INSTITUTE OF DENTAL RESEARCH

NIDR Research Digest

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Multiple Birth Defects Linked to Related Group of Proteins

A growing number of genetic disorders have been traced to a family of proteins that play a key role in the development of the human embryo. Scientists at Johns Hopkins University have identified several genetic mutations that disrupt the function of these proteins and produce far-reaching effects on the formation of the body, particularly the face, head, limbs, and skin. The disorders associated with the genetic mutations that affect this group of proteins are providing insights into the basic biological processes that link the development of seemingly unrelated parts of the body.

Dr. Ethylin Wang Jabs, director of the Johns Hopkins Center for Craniofacial Development and Disorders, has been studying the genetic mutations that affect proteins called fibroblast growth factor receptors (FGFR). These receptors are constituents of many adult and embryonic cell membranes, and their function in development is an area of active investigation. Disorders resulting from FGFR gene mutations indicate that the receptors can profoundly influence the formation of bone and skin. Through the efforts of Dr. Jabs and other research groups, nine genetic disorders are now attributable to over 50 different mutations in FGFR genes.

Beare-Stevenson Cutis Gyrata Syndrome

The newest disorder linked to an FGFR mutation was reported in a recent issue of *Nature Genetics*. In a study supported by the NIDR, Dr. Jabs and colleagues looked for FGFR gene abnormalities in five patients with the rare Beare-Stevenson Cutis Gyrata syndrome.

Individuals with this syndrome share certain common features, the most prominent of which is called cutis gyrata, for the furrowed skin that resembles the fine ribbing of corduroy. Other features include areas of excessive skin pigmentation, and severe abnormalities in the formation of the head and face, fingers and toes, and anal-genital areas. Affected individuals usually die in infancy, with the longest known survivor living 13 years.

The scientists focused their search on the genes for two fibroblast growth factor receptors, FGFR2 and FGFR3. Four FGFR genes have been identified so far, but numerous mutations have been found in specific regions of the FGFR2 and FGFR3 genes in individuals with syndromes that share features with Beare-Stevenson. The researchers identified two different mutations, each occurring in the same region of the gene that contains the molecular code for the FGFR2 protein. In three of the individuals examined, one or the other of the mutations was present, but not both. No mutations were found in the same regions of the FGFR2 or FGFR3 genes in the other two patients, indicating that mutations in other regions

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Announcing

The 1997 NIDR Summer Dental Student Award

"Frontiers in Oral Health Research"

Spend a Summer of Research
at the National Institute of Dental Research

A complete description of the program is on the NIDR web site:
http://www.nidr.nih.gov

Applications must be received by January 10, 1997

For further information contact the office of your dean, or:

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NIDR Funds Four Oral Cancer Research Centers

The NIDR, together with the National Cancer Institute, is stepping up the battle against oral cancer by funding centers for research that will investigate how oral cancer is triggered and what can be done to reduce its devastating effects. The four center grants, which total $2.8 million this year and will provide additional funds over a 5-year grant period, were awarded to the University of Alabama at Birmingham; the University of California, San Francisco; the University of Chicago and Northwestern University; and the M.D. Anderson Cancer Center at the University of Texas. All but the M.D. Anderson Cancer Center grant are co-funded by the National Cancer Institute.

The need for the centers is underscored by the fact that survival rates for oral cancer have not improved significantly

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Birth Defects Linked

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of these genes or in different genes may cause the syndrome in those individuals.

In addition to Beare-Stevenson Cutis Gyrata, mutations in the FGFR2 gene have been reported for Pfeiffer, Apert, Jackson-Weiss, and Crouzon syndromes. The common features shared by these disorders are an underdeveloped midface and craniosynostosis, or the premature fusing of the bony plates that make up the skull. Like Beare-Stevenson, some of these syndromes also produce abnormalities in the hands or feet, but skin problems are not usually involved.

Narrowing the Search

Dr. Jabs and other research groups are now tackling the complex problem of determining how specific mutations in the FGFR genes produce the spectrum of effects seen in the body. According to the investigators, the part of the receptor molecule that is affected by the mutation plays a key role.

Each member of the FGFR protein family consists of three basic regions; a component on the exterior of the cell that binds to molecular signals, a section that passes through the cell membrane (transmembrane region), and a section that protrudes into the cell's interior. When a signaling molecule binds to the receptor, it sets off a chain reaction that sends a message from the cell surface to the nucleus. The message can spur the cell to divide, migrate to another location, transform from an undifferentiated embryonic state to a specialized cell, or enter a cycle leading to cell death.

Overactive Receptors

According to Dr. Jabs and others, most FGFR mutations likely cause receptors to become overly active. The mutated receptors could bind to signals they would not normally recognize, or could 'misfire', sending a false message to the nucleus without having actually bound to a signal. The site of the mutation could affect the degree of receptor overactivity, the cell types that are affected, and ultimately, the array of anomalies seen throughout the body.

The two syndromes that share distinctive skin anomalies, Beare-Stevenson Cutis Gyrata and the unique cases of Crouzon, have FGFR2 and FGFR3 transmembrane mutations, respectively. The mutations occur on different genes yet appear to have a similar biologic effect on receptor function, disrupting both bone- and skin-forming cells. Mutations responsible for most of the related syndromes that lack skin manifestations occur in the external portion of the FGFR2 molecule, in or near the binding site. Unlike the transmembrane mutations, the binding site mutations primarily affect receptors on cells that form bone.

However, even slight differences in the location of an FGFR gene mutation can produce distinct syndromes. FGFR3 transmembrane mutations can also result in dwarfism, and mutations in the same region of the FGFR2 binding site produce distinct syndromes that vary in the degree of craniofacial, dental, and limb involvement. It is likely that environmental factors, or mutations in still other genes, may be important contributors to the diversity of anomalies that accompany mutations in this complex protein family.

Members of the research team were Dr. Kelly A. Przylepa, Mr. William Paznekas, Drs. Minghuang Zhang, Wilma Bias, and Ethylin Wang Jabs from the Johns Hopkins Center for Craniofacial Research; Dr. Mahin Golabi from the Division of Medical Genetics, University of California, San Francisco; Drs. Michael J. Bamshad and John C. Carey from the Division of Medical Genetics, University of Utah; Dr. Bryan D. Hall from the Division of Genetics/ Dysmorphology, University of Kentucky; Dr. Roger Stevenson from the Greenwood Genetic Center, Greenwood, South Carolina; Dr. Seth J. Orlow from the New York University School of Medicine; and Dr. M. Michael Cohen Jr. from Dalhousie University, Dalhousie, Halifax, Nova Scotia.

The study was supported through grants from the NIDR and the National Center for Research Resources at the National Institutes of Health, the Shriners Hospital for Crippled Children, and an Irma T. Hirschl Career Scientist Award. ■

—Wayne Little

Cancer Research Centers

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over the past 30 years. Oral cancer claims the life of one American every hour. Thirty thousand Americans are diagnosed with oral or pharyngeal cancer annually; only half survive more than five years.

Oral cancer usually occurs in people over age 40, but can develop at any age. It is twice as common in men as in women, and occurs more frequently in African Americans than in whites.

Multiple Factors At Work

The major risk factors for oral cancer are tobacco use, alcohol use, and—in the case of lip cancer—prolonged exposure to sunlight. "Oral cancer is a classic example of a disease that reflects the interaction of behavior, environment, and molecular genetics," explained NIDR Director Harold Slavkin. "Our goal is to reduce the behavioral and environmental risk factors for oral cancer, while we tackle what can be done at the molecular and genetic level to prevent or reverse the cancer process."

The new oral cancer research centers will take a multidisciplinary approach, including not only basic research, but also clinical investigations and rehabilitation to help patients return to normal activities. One of the projects will look at the effect of nutrition on the development of oral tumors. Another will focus on the role of viruses such as the human papillomavirus in causing oral cancer. Investigators will study the genetic basis for tumor growth, as well as identify markers for early diagnosis. Because oral cancer is complicated by the ability of cancerous cells to invade surrounding tissues and metastasize to distant sites in the body, the researchers also will explore the factors controlling this process at the molecular level.

New Therapies

The newly funded centers aim to develop improved treatments for oral cancer. Currently, the available treatments are surgery, radiation therapy, or in many cases, a combination of the two. Some patients also receive chemotherapy. Yet these treatments can have devastating effects. Disfigurement may result from the necessary surgery or radiation, and patients may experience long-term pain, dry mouth, and nerve dysfunctions that affect speech, chewing, swallowing, and taste perception.

"We need to move on to so-called 'smart' therapeutics," said Dr. Slavkin. "We need approaches that will not destroy healthy bone and salivary gland tissue adjacent to the cancer, but will enable us to replace defective genes or insert a 'suicide' gene to make malignant cells self-destruct." ■

NIDR Funds Studies Examining Health Effects of Dental Amalgams in Children

The NIDR will award more than $9 million in grants over the next five years to fund two clinical trials examining the health effects of dental amalgams in children. The studies, which are funded under a cooperative agreement between the NIDR and two grantee institutions, aim to document whether or not there are adverse health effects attributable to mercury-containing dental amalgams. Approximately 100 million people have amalgam fillings in the United States alone. Little is known about the health effects of low-level exposure to mercury because of the complexity of measuring exposure from multiple sources and assessing multiple potential effects.

Mercury is used in numerous industries, is found in foods, and is used to form various alloys, including dental amalgam. Dental amalgam, which is approximately 50 percent mercury, is the standard material used worldwide in restorations for the treatment of dental caries.

Some studies have suggested that mercury-containing dental amalgams may be the cause of various diseases ranging from mild skin conditions to debilitating neuromuscular diseases. Reports issued by a Food and Drug Administration panel in 1991 and the Public Health Service (PHS) in 1993 found no evidence suggesting harmful effects from amalgam *per se*, although they did stress the need for additional research to look for any possible long-term biological effects from the material. In 1995, following a meeting of government health officials from nine European nations and Canada, the PHS released a statement again reiterating that there are "no scientifically compelling reasons either to discontinue or to curtail the clinical use of dental amalgam or to recommend removal of exisiting amalgam fillings" without clear evidence of allergy or intolerance in individual patients. As part of an ongoing risk management program, the PHS is continuing to encourage research studies of amalgam safety as well as the development of non-mercury-containing dental filling materials.

The new clinical trials funded by the NIDR are large, multidisciplinary, multisite, prospective, and randomized. They are designed to provide answers to the ongoing safety debate.

One of the studies is a joint effort between teams of researchers at the University of Washington School of Dentistry and the University of Lisbon, Portugal. The study will focus on 500 Lisbon schoolchildren in need of dental restorations. The other study is a cooperative project involving scientists from the New England Research Institutes, Forsyth Dental Center, Harvard Children's Hospital, and the University of Rochester. This study will involve children at various sites in Boston and Maine.

While caries is a common experience among the children participating in the Lisbon study, most do not have access to dental care, notes principal investigator Dr. Timothy DeRouen, chair of Dental Public Health Sciences and professor of biostatistics at the University of Washington. The children, who range in age from 8 to 10 years old, attend a school that serves a disadvantaged population. The children were selected because they do not have a high exposure to mercury from seafood, they are in need of substantial dental care, and they can be easily tracked during the study period. Similarly, the caries rate of children in the Boston-Maine based study also is high, and these children, too, are in need of dental care.

While the two studies will be conducted independently and are sized for different clinical endpoints, both will use the same overall experimental design and a common set of clinical procedures and outcome measures. In both studies, one group of children will receive treatment with dental amalgam, and the other group will receive a different restorative material. To assess any possible health effects, teams of experts in dentistry, toxicology, psychology, nephrology, oral medicine, and neurology will administer extensive behavioral, renal, and neurological tests before the children receive dental treatment and on several occasions afterwards. Urine samples also will be collected on a regular basis and examined for mercury levels. At the Boston and Maine sites, in addition to evaluating the neurological and behavioral outcome measures common to both studies, researchers also will look for any changes in the children's IQ resulting from low-level mercury exposure. All study participants will be followed for five years. ■

NIDR to Sponsor Grantsmanship Workshop at IADR/AADR Meeting

To provide insight into what makes a grant application both scientifically strong and exciting to read, the NIDR will sponsor a workshop on "Contemporary Grantsmanship," Wednesday, March 19 at 1:15 p.m. during the annual meetings of the AADS and IADR/AADR in Orlando, Florida.

Seeking financial support for research is a challenging activity that has become a major responsibility for scientists. All applicants for NIDR funding—especially post-doctoral students and new investigators—are encouraged to attend this workshop. The featured speakers are representatives from NIDR's Division of Extramural Research.

Participants in the workshop will learn about the characteristics of a successful grant application, the review process that is used at NIH to evaluate the quality of applications, and how funding decisions are made. The rules and regulations that must be met when preparing an application to the Federal government, or accepting a grant, also will be discussed. Information will be provided about whom to contact for answers to specific questions.

The workshop is free and open to all.

Conference on Microbial Ecology and Infectious Disease Draws Overflow Crowd

Nobel Laureate Joshua Lederberg was among the prominent speakers who addressed the more than 325 people attending the "Microbial Ecology and Infectious Disease" conference, recently held in Bethesda, Maryland. The conference was cosponsored by the NIDR, the National Institute of Allergy and Infectious Diseases, and the Center for Biologics Evaluation and Research. Dr. Lederberg described the relationship between a deteriorating global environment and increasing population size, and how this is providing rich opportunities for new and reemerging pathogens to establish major footholds throughout the world. Observations reported by other researchers supported the probability of such scenarios occurring, especially since the main line of defense against invasive pathogens has been dramatically weakened by the widespread use of antibiotics.

Among the other topics of discussion were various invasion motifs exhibited by invasive bacteria, the diversity of mechanisms by which bacteria attach to host cells, bacteria-to-bacteria communication, the reception of environmental signals, and the ecology of specific pathogens. ■

NIDR Pain Research Honored

The American Pain Society presented its highest honors in pain research to two investigators for their work at the NIDR. Drs. Mitchell Max and Gary Bennett received the awards, each accompanied by a $10,000 prize, at the Society's annual meeting, held November 14-17 in Washington, D.C.

Dr. Max, chief of the NIDR clinical trials unit, Pain and Neurosensory Mechanisms Branch, received the Wilbert E. Fordyce Clinical Investigator Award for developing innovative clinical trial methods and treatments for pain caused by nerve damage. His research has focused on the mechanisms and treatment of pain caused by conditions such as postherpetic neuralgia (nerve damage that can follow an attack of shingles) and diabetic neuropathy.

Dr. Bennett received the Frederick W. L. Kerr Basic Science Research Award for his development of an animal model of chronic neuropathic pain that has been adopted by scientists at universities and pharmaceutical companies around the world and has led to the development of investigational drugs for treating persistent pain. The award also recognized his contributions to defining the peripheral and central mechanisms by which nerve injury leads to chronic pain. Until this past July, Dr. Bennett was chief of NIDR's neuropathic pain and pain measurement section in the Pain and Neurosensory Mechanisms Branch. He is now professor of neurology at the Allegheny University of the Health Sciences in Philadelphia. ■

Dr. Mitchell Max addresses attendees at the annual meeting of the American Pain Society after receiving the Wilbert E. Fordyce Clinical Investigator Award.

The *NIDR Research Digest* is published periodically by the National Institute of Dental Research. Its purpose is to communicate NIDR research findings and other activities of interest to those working in the field of dental research.

Requests for additional information on NIDR or its programs may be addressed to: NIDR, Public Information and Reports Branch, 31 Center Drive MSC 2290 Bethesda, MD 20892-2290. Telephone (301) 496-4261.